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Anti-arteriosclerotic and Anti-hyperlipidemic Effects of Sea Mustard (*Undaria pinnatifida*) in Sprague-Dawley rats

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This study investigated the inhibitory effects of sea mustard on high-fat diet-induced obesity and hyperlipidemia in Sprague-Dawley rats. Sea mustard (*Undaria pinnatifida*) powder, sea mustard ethanol extract, and sea mustard ethanol-extracted residue were tested. The ethanol extracted residue had the most beneficial anti-hyperlipidemic activity. Alginate in the sea mustard was considered to be the key component. The ethanol-extracted residue of sea mustard also had antioxidant activity, which may be effective in preventing hyperlipidemia by increasing the enzymatic activity of superoxide dismutase, which can remove active oxygen from the bloodstream.

Key words: Anti-obesity, Antioxidant, Anti-hyperlipidemic, Alginate

Introduction

Improved standards of living have led to increased intake of saturated animal fat, which is associated with a variety of health disorders. As a result of nutritional imbalance, the occurrence of circulatory diseases, including high blood pressure and arteriosclerosis, has increased. The most noticeable change has been an increase in the proportion of cholesterol in the form of low-density lipoprotein (LDL), which can account for 60 to 70% of total cholesterol. High levels of LDL cholesterol may result from a deficiency of LDL receptors, an excess of apolipoprotein (Apo), or an increase of chylomicrons and very low-density lipoproteins (VLDL), which are induced by a diet rich in triglycerides (Hong et al., 1998). Keith (1991) reported that hypercholesterolemia is clinically recognized as the most important atherosclerosis risk factor. Dietary fiber is effective in preventing and reversing circulatory system diseases caused by a high-fat diet, because fiber hinders the absorption of cholesterol and neutral fats (Spiller and Amen, 1975). In the past, seaweed has not been considered as a valuable dietary component; it contains large amounts of indigestible polysaccharides and therefore is a poor energy source. However, recent research has suggested that eating seaweed may have health benefits (Kim et al., 1988; Lee, 1996; Park et al., 1996; Kim and Kim, 1998). Seaweed has been shown to contain soluble fiber that can lower serum lipid levels (Kim et al., 1988), and this finding has prompted much research interest.

Sea mustard, a type of seaweed, is an alkaline health food that contains abundant active physiological substances such as Na, K, Ca, Mg, P, S, dietary fiber, linoleic acid, and vitamins (Anderson and Trityen-Clark, 1986; Anderson and Gustafson, 1988). In particular, alginic acid, the mucopolysaccharide in sea mustard, is effective in discharging heavy metal and radioactive substances from the body and in decreasing cholesterol, as well as preventing obesity, constipation, high blood pressure, and glycosuria (Choi et al., 1991; Kim and Kim, 1982; Kim and Cheong, 1984). Fucoidan extracted from sea mustard may also have beneficial effects with regard to cancer, cholesterol, blood coagulation, blood pressure, and lipid metabolism. Although sea mustard contains other biologically active components, including calcium and mucopolysaccharides, fucoidan is the component that hinders the growth of tumor cells such as sarcoma 180, L-1210, meth A, and B-16 melanoma (Sato et al.,

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2002; Suetsuna et al., 2004; Yamamoto et al., 1984; Yamamoto et al., 1981).

In this study, we verified the effects of sea mustard on hyperlipidemia in Sprague-Dawley rats. We administered sea mustard powder, an ethanol extract of sea mustard, and the ethanol-extracted residue of sea mustard to evaluate their anti-hyperlipidemic, anti-arteriosclerotic, and antioxidant activities.

Materials and Methods

Sea mustard sample preparation

Sea mustard (*Undaria pinnatifida*) used in this study was collected and dried by Donghae Seaside, and was purchased from Chumunjin fish market in Korea. Animals were administered dried sea mustard powder, its ethanol extract, or the ethanol-extracted residue. To prepare the extract and residue, 100 g of dried sea mustard powder was mixed with 500 mL of 95% ethanol (5-fold the sample weight) in a 1,000 mL Erlenmeyer flask, followed by sonication (Powersonic 520; Hwashin, Korea) for 30 min at 30°C. The extract was filtered through Whatman No. 2 filter paper, concentrated, frozen, dried on a rotary evaporator (Rotavator R-200; Buchi, Switzerland), and stored at -40°C.

Experimental animals

Male Sprague-Dawley rats (140±10 g), provided by Hyochang Science, Daegu, Korea, were divided into six groups of five rats each. The animals were housed at a relative humidity of 50±10% and a temperature of 22±3°C, with a 12 h light (07:00-19:00)/dark cycle throughout the experiment (Tecniplast, Italy). A 1-week adaptation period was allowed before the experiment began, and only healthy animals were used for the experiment. The rats were provided with a normal basal diet, or a diet that caused hyperlipidemia along with a sea mustard preparation. Water was provided freely, but diet intake was measured. Each rat was orally administered 200 mg/kg of a sea mustard sample every day for 6 weeks. During 24 h before the experiment, only water was offered to the animals. Animals were sacrificed at a fixed time (10:00-12:00).

Induction of hyperlipidemia by a high-fat diet

Compositions of the normal and high-fat diets used in this study are shown in Table 1. Preliminary experiments had demonstrated that hyperlipidemia was induced in the rats after 6 weeks of the high-fat diet. The rats were fasted for 8 h after the final

Table 1. Composition of basal and hyperlipidemic diet

Ingredient	Basal diet (%)	Hyperlipidemic diet (%)
Casein	20.0	20.0
DL-Methionine	0.3	0.3
Corn starch	15.0	15.0
Sucrose	50.0	34.5
Fiber[1)]	5.0	5.0
Corn oil	5.0	-
AIN-mineral mixture[2)]	3.5	3.5
AIN-vitamin mixture[3)]	1.0	1.0
Choline bitartate	0.2	0.2
Beef tallow	-	20.5

[1)]Cellulose : Sigma Co. LTD., USA.

[2)]Mineral mixture based on the pattern of Rogers and Haper (1965) contain the following (g/kg diet): calcium phosphate dibasic 500.0, sodium chloride 74.0, potassium citrate monohydrate 220.0, potassium sulfate 52.0, magnesium oxide 24.0, magnesium carbonate 3.5, ferric citrate 6.0, zinc carbonate 1.6, cupuric carbonate 0.3, potassium iodate 0.01, chromium potassium sulfate 0.55, sucrose, finely powered make 1,000.

[3)]Vitamin mixture(g/kg diet): thiamine HCl 0.6, biotin 0.02, riboflavin 0.6, cyanocobalamine 0.001, pyridoxine HCl 0.7, retinyl acetate 0.8, nicotinic acid 3.0, DL-tocopherol 3.8, Ca-pantothenate 1.6, 7-dehydrocholesterol 0.0025, folic acid 0.2, methionine 0.005, sucrose, finely powered make 1,000.

treatment and then anesthetized with CO_2. Blood samples were collected from the abdominal aorta [the heart] using non-heparinized syringes and incubated in ice water for 30 min. Serum was separated by centrifugation at 3,000 rpm for 10 min at 4°C and preserved at -70°C.

Measurement of body and fatty tissue weights

Body weight was measured every week throughout the experiment. Abdominal fat pad weight was determined by weighing the retroperitoneal and epididymal adipose tissues removed after the animals were sacrificed.

Measurement of lipids in hepatic tissue and feces

After being removed and weighed, hepatic tissues were kept in a -70°C freezer, and 4 days of feces were collected. Neutral fat and cholesterol were extracted according to Folch et al. (1957).

Measurement of total blood lipid content

Total blood lipids were measured using a colorimetric method that relies on sulfo-phospho-vanillin reaction principles (Fringe and Dunn, 1980). Samples were heated with sulfuric acid and reacted with vanillin phosphoric acid, leading to a loss of

pink color detectable at 540 nm. The results are expressed as milligrams per deciliter.

Measurement of total cholesterol level

Total serum cholesterol was measured using an AM 202-K assay kit according to the manufacturer's instructions (Asan Pharm. Co., Korea) and the method of Richmond (1976). Briefly, 3 mL of assay solution (cholesterol esterase 20.5 U/L, cholesterol oxidase 10.7 U/L, sodium hydroxide 1.81 g/L, potassium phosphate monobasic 13.6 g/L, phenol 1.88 g/L) were mixed with 20 μL of serum sample and incubated at 37°C for 5 min. The absorbance of the reaction mixture was measured at 500 nm, and the total cholesterol level was calculated from a standard calibration curve.

Measurement of triglyceride level

Triglycerides were measured using an AM 157S-K assay kit according to the manufacturer's instructions (Asan Pharm. Co., Korea) and the method of McGowan et al. (1983). Briefly, 3 mL of assay solution (lipoprotein lipase 10,800 U, glycerol kinase 5.4 U, peroxidase 135,000 U, L-α-glycero phosphooxidase 160 U, N,N-bis(2-hydroxyethyl)-2-aminomethane sulfonic acid 0.427 g/dL) were mixed with 20 μL of serum sample and incubated at 37°C for 10 min. The absorbance of the reaction mixture was measured at 550 nm, and the total triglyceride level was calculated from a standard calibration curve.

Measurement of phospholipid level

Phospholipids were measured using an assay kit according to the manufacturer's protocol (Iatron Chem. Co., Tokyo, Japan) and the method of Chen et al. (1979).

Measurement of high-density lipoprotein (HDL)-cholesterol level

Serum HDL-cholesterol was measured using an AM 203-K assay kit according to the manufacturer's protocol (Asan Pharm. Co., Korea) and the method of Noma et al. (1986). Briefly, 20 μL of serum sample were mixed with 200 μL of a solution containing 0.1% dextran sulfate and 0.1 M magnesium chloride, and incubated at room temperature for 10 min After centrifugation at 2,500×g for 10 min, 100 μL of the supernatant were mixed with 3 mL of assay solution (lipoprotein lipase 10,800 U, glycerol kinase 5.4 U, peroxidase 135,000 U, L-α-glycero phosphooxidase 160 U, N,N-bis(2-hydroxyethyl)-2-aminomethane sulfonic acid 0.427 g/dL) and incubated at 37°C for 5 min. The absorbance of the reaction mixture was measured at 500 nm, and the results were calculated in milligrams per deciliter from a standard calibration curve.

Calculation of low density lipoprotein (LDL)-cholesterol level

The LDL-cholesterol level was calculated using the equation of Friedewald et al. (1972):

LDL cholesterol=total cholesterol−
(HDL-cholesterol + triglycerides/5).

Measurement of lipid peroxidation

Lipid peroxidation was measured in serum, according to the method of Yagi (1987). Serum was preincubated with 1/12 N HSO and 10% phosphortungstic acid for 5 min, and then centrifuged. The protein pellet obtained was resuspended in 1/12 N HSO and 10% phosphotungstic acid, and the procedure was repeated. The protein pellet was then suspended and incubated in 1 mL of distilled water with 0.67% thiobarbituric acid and 50% acetic acid at 95°C for 60 min, after which 5 mL of n-BuOH were added, and the mixture was allowed to stand at room temperature. The red n-BuOH layer was separated by centrifugation for 10 min, and its absorbance was measured using a fluorometer (excitation, 515 nm; emission, 553 nm). The malondialdehyde (MDA) concentration (nmol/mL of serum) was calculated from a standard curve.

Measurement of hydroxyl radicals

Hydroxyl radicals were measured in serum according to the method of Kobatake et al. (1987). Briefly, 333.3 μL of a mixture composed of 34.8 μL of serum, 0.54 M NaCl, 0.1 M potassium phosphate buffer (pH 7.4), 10 nM NaN_3, 7 nM deoxyribose, 5 nM ferrous ammonium sulfate, and distilled water was mixed on a vortexer and incubated at 37°C for 15 min. After 67 μL of this sample were mixed with 75 μL of 8.1% sodium dodecyl sulfate, 67 μL of 20% acetic acid, and distilled water, 222 μL of 1.2% thiobarbituric acid were added. The final solution was boiled for 30 min, cooled to room temperature, and clarified by centrifugation at 700×g for 5 min. Absorbance of the supernatant was determined at 532 nm, and the concentration of hydroxyl radical (nmol/mg protein) was calculated from a standard curve.

Measurement of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined in serum using the method of Oyanagui (1984). Serum was diluted 100-fold with potassium phosphate buffer,

and 100 μL of the diluted sample were placed into a test tube. Then, 200 μL of solution A (3 nM hydroxylamine/3 nM hypoxanthine), 200 μL of solution B (7.5 mU/mL xanthine oxidase with 0.1 mM EDTA-Na_2), and 500 μL of distilled water were added. The solutions were mixed on a vortexer and incubated at 37°C for 40 min. Finally, 2 mL of solution C (300 mg of sulfanilic acid/5.0 mg N-1-naphtyl-ethylenediamine in 500 mL of 16.7% acetic acid) were added, and the mixture was allowed to stand at room temperature for 20 min. The absorbance of the sample was measured at 550 nm, and SOD activity was calculated from a standard curve.

Measurement of protein content

Protein content was determined by the method of Lowry et al. (1951).

Statistical analysis

Means and standard deviations of data from individual experiments were calculated. Significant differences between individual experiments were determined using Duncan's multiple range test. Values of $p \leq 0.05$ were taken to indicate statistical significance.

Results and Discussion

Anti-obesity effect

Table 2 shows body weight changes in the rats with diet-induced hyperlipidemia after the addition of sea mustard to their diets. In rats receiving sea mustard ethanol extract, the weight decreased in weeks 1 and 2. By week 6, body weight had declined in rats fed all three sea mustard preparations (powder, ethanol extract, and ethanol-extracted residue) compared with the control group. The rate of weight decrease was greatest for the rats receiving ethanol-extracted residue.

This finding suggests that the anti-obesity properties of sea mustard are not attributable exclusively to ethanol-soluble components, but rather it is likely that the fiber in the ethanol-extracted residue also contributed to the anti-obesity effect. This is contrary to results reported by Maeda et al. (2005), who found that fucoxanthin in sea mustard was responsible for its anti-obesity properties. On the other hand, Lee et al. (1996) reported that a group of diabetic rats fed sea mustard showed a tendency toward weight gain, compared with the diabetic control group.

Fluctuation of fatty tissue weight

Table 3 shows the effects of the sea mustard preparations on the weight of fatty tissues in the rats. The quantities of retroperitoneal lipids in rats fed sea mustard powder, ethanol extract, and ethanol-extracted residue were 12.3±1.3, 14.5±2.4, and 10.3±1.4 mg/g, respectively, compared with the control group (14.9±2.1). On the other hand, the weight of epididymal lipids in rats fed ethanol-

Table 2. Effect of sea mustard on the body weight in rats fed a normal and hyperlipidemic diet for 6 weeks

Treatment	Dose (mg/kg)	1	2	3	4	5	6 (Weeks)
		Body weight gain (g)					
Normal		69.7±8.3^{b}	117.9±10.6^{b}	138.5±19.4^{e}	181.7±29.6^{b}	211.7±27.2^{f}	220.8±28.6^{f}
Control		88.5±9.5^{a}	161.3±21.3^{a}	221.7±30.5^{abc}	251.2±21.1^{a}	306.8±20.5^{ab}	331.7±31.7^{a}
Sea mustard powder	200	83.9±7.2^{a}	165.5±19.8^{a}	198.3±18.6^{bcd}	236.5±20.6^{a}	281.3±19.1^{bcde}	293.6±24.5^{bcde}
Ethanol extracts	200	81.8±8.6^{a}	154.7±18.7^{a}	210.4±17.2^{abcd}	249.7±19.4^{a}	294.8±18.3^{abcd}	300.7±19.8^{bcd}
Ethanol extracted residues	200	90.3±9.4^{a}	160.2±20.8^{a}	195.6±21.3^{cd}	226.9±18.8^{a}	274.5±19.5^{cde}	281.2±18.6^{de}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

Table 3. Abdominal fat pad weight in the normal and fat diet-induced rats fed sea mustard for 4 weeks

Treatment	Dose (mg/kg)	Retroperitoneal	Epididymal
		mg/g body weight	
Normal		6.3 ± 3.2^{f}	7.8 ± 2.0^{f}
Control		14.9 ± 2.1^{a}	12.4 ± 1.4^{abc}
Sea mustard powder	200	12.3 ± 1.3^{bcd}	11.9 ± 1.0^{abcd}
Sea mustard ethanol extracts	200	14.5 ± 2.4^{bc}	13.1 ± 1.1^{a}
Ethanol extracts sea mustard residues	200	10.3 ± 1.4^{de}	10.4 ± 1.0^{de}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

extracted residue was 10.4±1.0 mg/g, which is 16% less than that of the control group (12.4±1.4 mg/g). These results indicate that ethanol-extracted residue is more effective than the other two preparations in reducing lipid storage, probably owing to its fiber content, which includes alginic acid.

Change of serum lipid components

Table 4 shows the concentrations of lipid components in the sera of rats fed the sea mustard preparations. In rats fed ethanol-extracted residue, the concentration of phospholipids was 131.3±11.5 mg/dL, which is a decrease of 11.7% compared with the control group (148.7±10.2 mg/dL). Serum triglyceride concentrations in the rats fed sea mustard powder and ethanol-extracted residue were 181.6±11.8 mg/dL and 167.4±12.5 mg/dL, respectively, which are 9.3% and 16.4% lower, respectively, than the control value (200.3±12.4 mg/dL).

George et al. (1982) reported that fiber combining with bile salts inhibits lipid absorption and directly accounts for most of the decrease in lipid levels caused by fiber intake. Akiba and Matsumoto (1982) reported that fiber decreases the concentration of serum phospholipids. Furthermore, Goode et al. (1995) found that the lipid content of the serum has an intimate interrelation with blood pressure, and that patients with high blood pressure have a higher total lipid content in the serum. The results of this experiment suggest that a sea mustard diet may be effective in improving hyperlipidemia.

Changes of serum cholesterol content and atherosclerosis indices

Table 5 shows the effect of the sea mustard preparations on serum cholesterol content and atherosclerosis indices in the rats. The serum total cholesterol concentration in rats with diet-induced hyperlipidemia was 91.6±5.2 mg/dL. However, total cholesterol decreased to 83.6±6.1 mg/dL (8.7% decrease) or 76.8±4.3 mg/dL (16.2% decrease) in rats fed sea mustard powder or ethanol-extracted residue, respectively. Conversely, HDL-cholesterol increased to 35.6±1.2 mg/dL, an 11.9% increase from t the control group (31.8±1.2 mg/dL). LDL-cholesterol was 7.6±0.9 mg/dL in rats with diet-induced hyperlipidemia. After administering sea mustard powder, ethanol extract, or ethanol-extracted residue, it had decreased to 7.2±0.8, 7.5±0.7, or 6.7±0.7 mg/dL, respectively. It has been reported that fiber decreases lipid absorption in the intestine and increases bile acid excretion, while also promoting bile acid synthesis from cholesterol, thus further decreasing the cholesterol concentration. Short-chain fatty acids, which are produced and act in the large intestine, hinder the synthesis of endogenous cholesterol (Chen and Anderson, 1979; Story, 1981; Anderson and Bridges, 1984; Nishina and Freedland, 1990; Schrijver et al., 1992). In particular, water soluble fibers such as pectin, gums, mucilages, and sea algae polysaccharides decrease the concentrations of total cholesterol and LDL-cholesterol, while increasing the concentration of HDL-

Table 4. Effect of sea mustard on the concentration of serum lipids in rats fed a hyperlipidemic diet for 4 weeks

Treatment	Dose (mg/kg)	Phospholipid	Triglyceride
		mg/dL	
Normal		120.4 ± 14.6^{d}	68.7 ± 7.26^{f}
Control		148.7 ± 10.2^{a}	200.3 ± 12.4^{a}
Sea mustard powder	200	138.8 ± 11.7^{abc}	181.6 ± 11.8^{bc}
Ethanol extracts	200	142.5 ± 12.3^{abc}	190.5 ± 10.7^{ab}
Ethanol extracted residues	200	131.3 ± 11.5^{bcd}	167.4 ± 12.5^{de}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

Table 5. Effect of sea mustard on serum cholesterol and AI in rats fed a hyperlipidemic diet for 4 weeks

Treatment	Dose (mg/kg)	Cholesterol (mg/dL)			
		Total	HDL	LDL	AI
Normal		56.8 ± 6.3^{g}	41.6 ± 2.4^{a}	2.6 ± 0.6^{a}	0.4 ± 0.1^{h}
Control		91.6 ± 5.2^{a}	31.8 ± 1.2^{f}	7.6 ± 0.9^{b}	1.9 ± 0.2^{a}
Sea mustard powder	200	83.6 ± 6.1^{cd}	35.4 ± 2.2^{def}	7.2 ± 0.8^{bc}	1.4 ± 0.1^{de}
Ethanol extracts	200	90.5 ± 4.2^{ab}	33.5 ± 1.2^{def}	7.5 ± 0.7^{b}	1.7 ± 0.1^{b}
Ethanol extracted residues	200	76.8 ± 4.3^{ef}	35.6 ± 1.2^{bc}	6.7 ± 0.7^{a}	1.2 ± 0.1^{f}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$). AI(Atherosclerosis Index) = (total cholesterol - HDL cholesterol) / HDL cholesterol.

cholesterol (Yang et al., 1996; Park et al., 1994; Kang et al., 1994; Fernandez et al., 1990). Furthermore, it has been reported that a diet including a sea mustard or laver mixture remarkably decreased serum cholesterol in diabetic rats (Lee et al., 1996). In particular, diets containing carrageenan, a water-soluble polysaccharide found in red algae, have been found to decrease cholesterol concentrations (Tsai et al., 1976; Jang and Park, 1995).

Our study demonstrated an effect on total cholesterol caused by ingestion of sea mustard powder and sea mustard ethanol extract, and HDL- and LDL-cholesterol levels were modified by consumption of ethanol-extracted residue. These results show that the addition of sea mustard is effective in improving hyperlipidemia. Moreover, atherosclerosis indices in rats fed sea mustard powder, ethanol extract, and ethanol-extracted residue were 1.4±0.1, 1.7±0.1, and 1.2±0.1 mg/dL, representing decreases of 27.6%, 9.6%, and 38.8%, respectively, compared with rats having diet-induced hyperlipidemia (index, 1.9±0.2 mg/dL).

Changes of lipid and cholesterol contents of hepatic tissue

When cholesterol is fed to rats, the serum free cholesterol concentration increases (Chung et al., 1988). On the other hand, the total cholesterol/cholesteryl ester consistency ratio is normally 64-72% in humans, but it declines with hepatotoxicity and rises with hypercholesterolemia (Jang and Park, 1995). Excess fatty acids from a high-fat diet are converted into triglycerides in the liver, and when the hepatic lipids are not removed, a fatty liver condition can develop (Yang et al., 1996). The cholesterol concentration in the liver is an important indicator of circulatory system diseases, as the liver delivers cholesterol to the blood in the form of soluble or ester-type cholesterol (Choi et al., 1987). Table 6 shows the effect of the sea mustard preparations on the hepatic lipid concentration and cholesterol content in rats with diet-induced hyperlipidemia. The total lipid concentrations in rats fed sea mustard powder and ethanol-extracted residue were 31.4±1.4 mg/g (10% lower than control) and 30.2±1.2 mg/g (13.5% lower than control), respectively. The triglyceride level in rats fed ethanol-extracted residue was 24.3±1.8 mg/g, 7.9% lower than the control value (26.4±2.1 mg/g). These results imply that the addition of sea mustard to the diet can affect the disease state of an animal, indirectly demonstrating its effect on hyperlipidemia.

Change of MDA concentration in hepatic tissue

Table 7 shows the effect of the sea mustard preparations on lipid peroxidation, measured as

Table 6. Effect of sea mustard on the hepatic lipid concentration of hepatic tissue of rat fed a hyperlipidemic diet for 4 weeks

Treatment	Dose (mg/kg)	Total lipid	Triglyceride	Cholesterol
		mg/g of tissue		
Normal		16.8 ± 2.0^{f}	9.4 ± 1.0^{d}	2.6 ± 0.5^{b}
Control		34.9 ± 2.2^{a}	26.4 ± 2.1^{a}	6.3 ± 0.4^{a}
Sea mustard powder	200	31.4 ± 1.4^{cd}	25.2 ± 2.0^{abc}	6.2 ± 0.5^{a}
Ethanol extracts	200	33.7 ± 1.3^{ab}	26.2 ± 1.7^{ab}	6.2 ± 0.5^{a}
Ethanol extracted residues	200	30.2 ± 1.2^{de}	24.3 ± 1.8^{bc}	6.0 ± 0.6^{a}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

Table 7. Effect of sea mustard on thiobarbituric acid(TBARS) concentration of hepatic homogenate of rats fed hyperlipidemic diet for 4 weeks

Treatment	Dose (mg/kg)	Thiobarbituric acid (TBARS)
		MDA nmol/mL serum
Normal		26.9 ± 3.6^{h}
Control		52.4 ± 2.2^{a}
Sea mustard powder	200	45.8 ± 3.1^{de}
Ethanol extracts	200	49.6 ± 2.6^{ab}
Ethanol extracts sea mustard residues	200	40.7 ± 2.0^{f}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

thiobarbituric acid reactive substances (TBARS) and

Table 8. Effect of the sea mustard on the serum hydroxyl radical and superoxide dismutase(SOD) activities in rats fed hyperlipidemic diet 4 weeks

Treatment	Dose (mg/kg)	Hydroxy radical	SOD activity
		nmol/mg protein	Unit/mg protein
Normal		3.0 ± 0.4^{e}	3.5 ± 0.3^{a}
Control		5.9 ± 0.3^{a}	1.7 ± 0.2^{bcd}
Sea mustard powder	200	5.2 ± 0.2^{cd}	1.8 ± 0.1^{bcd}
Ethanol extracts	200	5.7 ± 0.3^{ab}	1.7 ± 0.1^{bcd}
Ethanol extracts sea mustard residues	200	5.2 ± 0.3^{cd}	1.8 ± 0.2^{bc}

Values are mean±S.D. for six experiments. Values followed by the same letter are not significantly different ($P<0.05$).

Unit: on unit of SOD was defined as the which inhibited the reduced of alkaline.

DMSO-mediated adrechrome by 50% in one ml of blood.

Thiobarbituric acid reactive substances (TBARS) and expressed as MDA equivalents, in hepatic tissue of rats with diet-induced hyperlipidemia. The MDA concentrations in rats fed sea mustard powder (45.8± 3.1 nmol/mL) and ethanol-extracted residue (40.7± 2.0 nmol/mL) were 12.6% and 22.3% lower the control value (52.4±2.2 nmol/mL), respectively. Lipid peroxidation produces cell damage and may contribute to cancer, atherosclerosis, and aging (Cho and Bang, 2004). The increased oxidative stress associated with lipid peroxidation in tissues may also be reduced by the consumption of sea mustard. Increases in the MDA content of kidney and liver have been reported by Celik et al. (2002), and Velthuis-te et al. (1996) have reported similar findings.

Change of serum hydroxyl radical and superoxide dismutase activities

Table 8 shows the effect of the sea mustard preparations on serum hydroxyl radical and superoxide dismutase activities in the rats. The hydroxyl radical concentration in the groups fed sea mustard powder and ethanol-extracted residue was 5.2 nmol/mg, which was 12% lower than that in the control group (5.9±0.3 nmol/mg). Superoxide dismutase activity was notably different between the control group and the group fed sea mustard ethanol extract; however, no effects were observed in the rats fed sea mustard powder or ethanol-extracted residue.

Our results suggest that the ethanol-extracted residue of sea mustard may be a functionally beneficial food owing to its anti-obesity, antioxidant, anti-hyperlipidemic, and anti-arteriosclerotic effects.

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